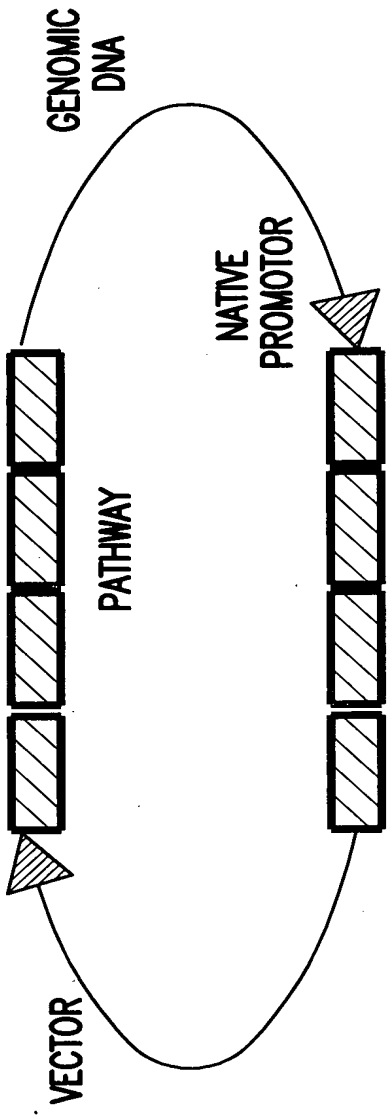


FIG.1

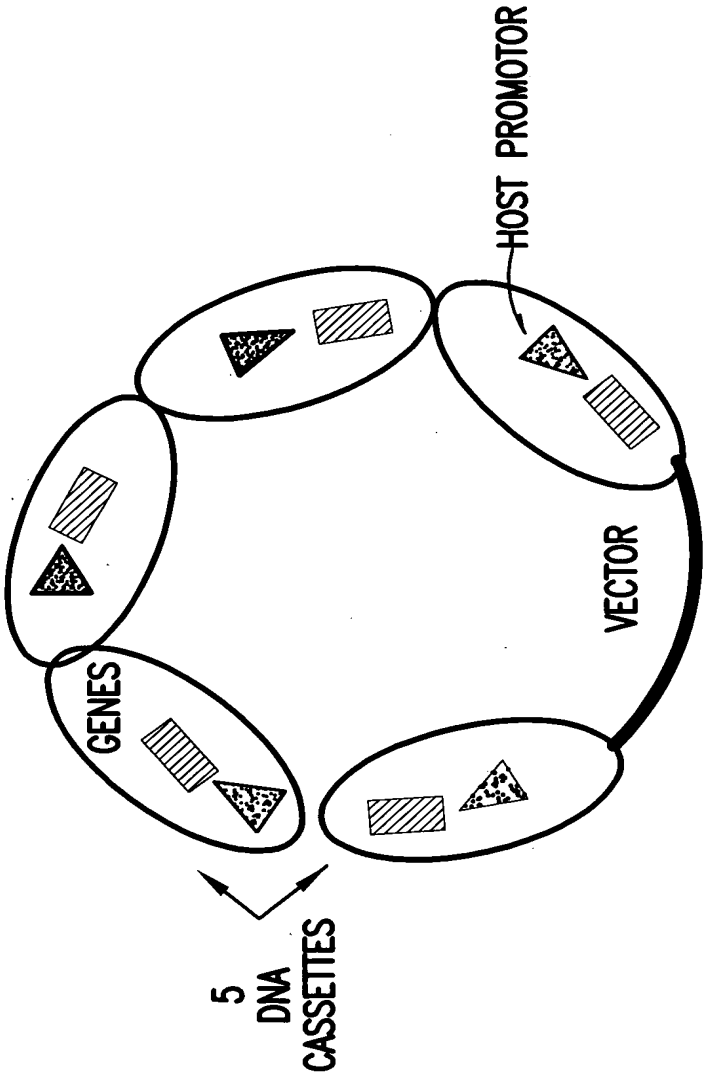


FIG.2

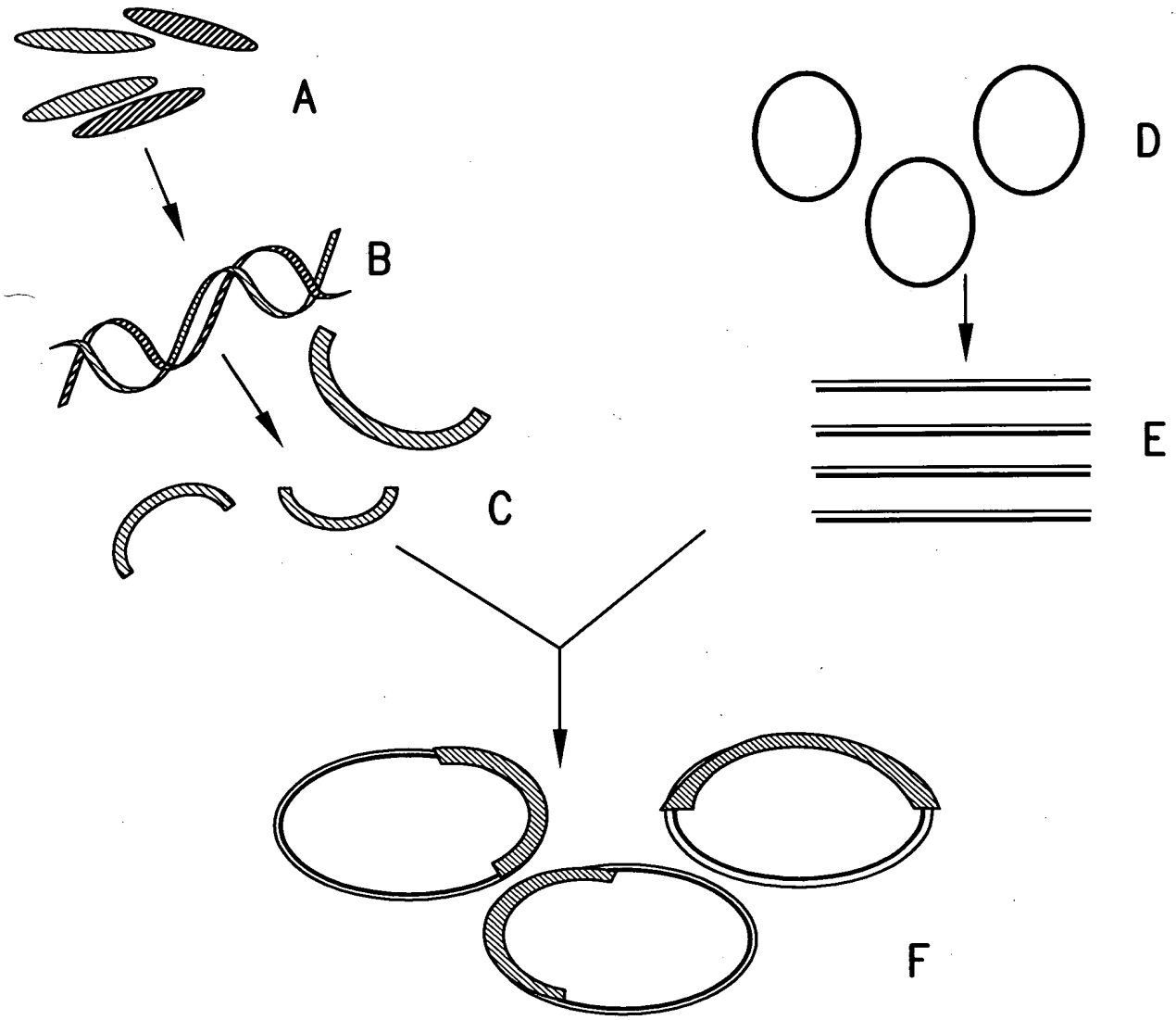


FIG.3

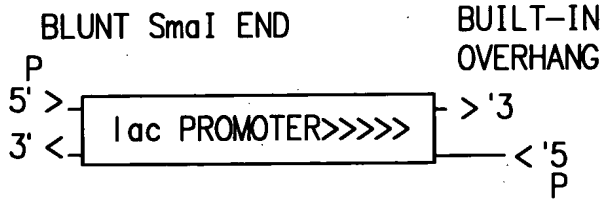


FIG.4A

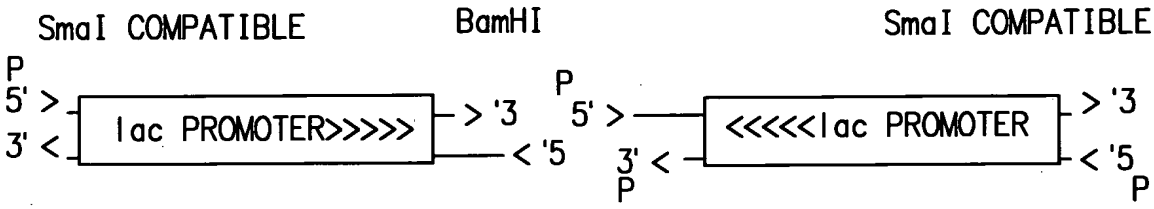


FIG.4B

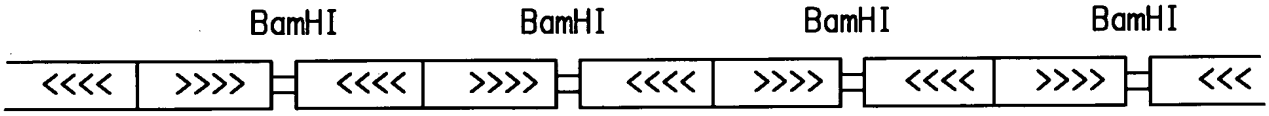


FIG.4C

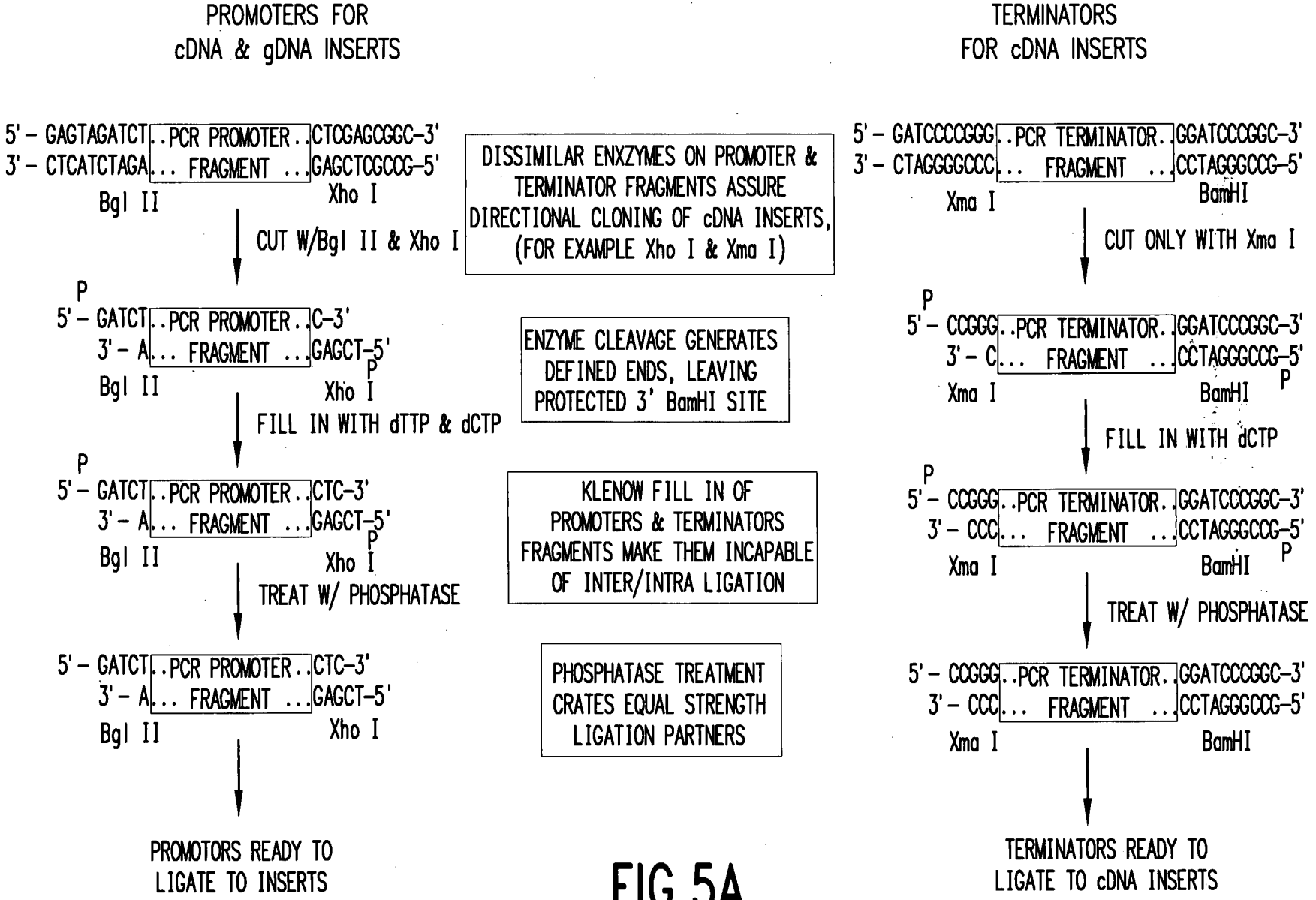


FIG.5A

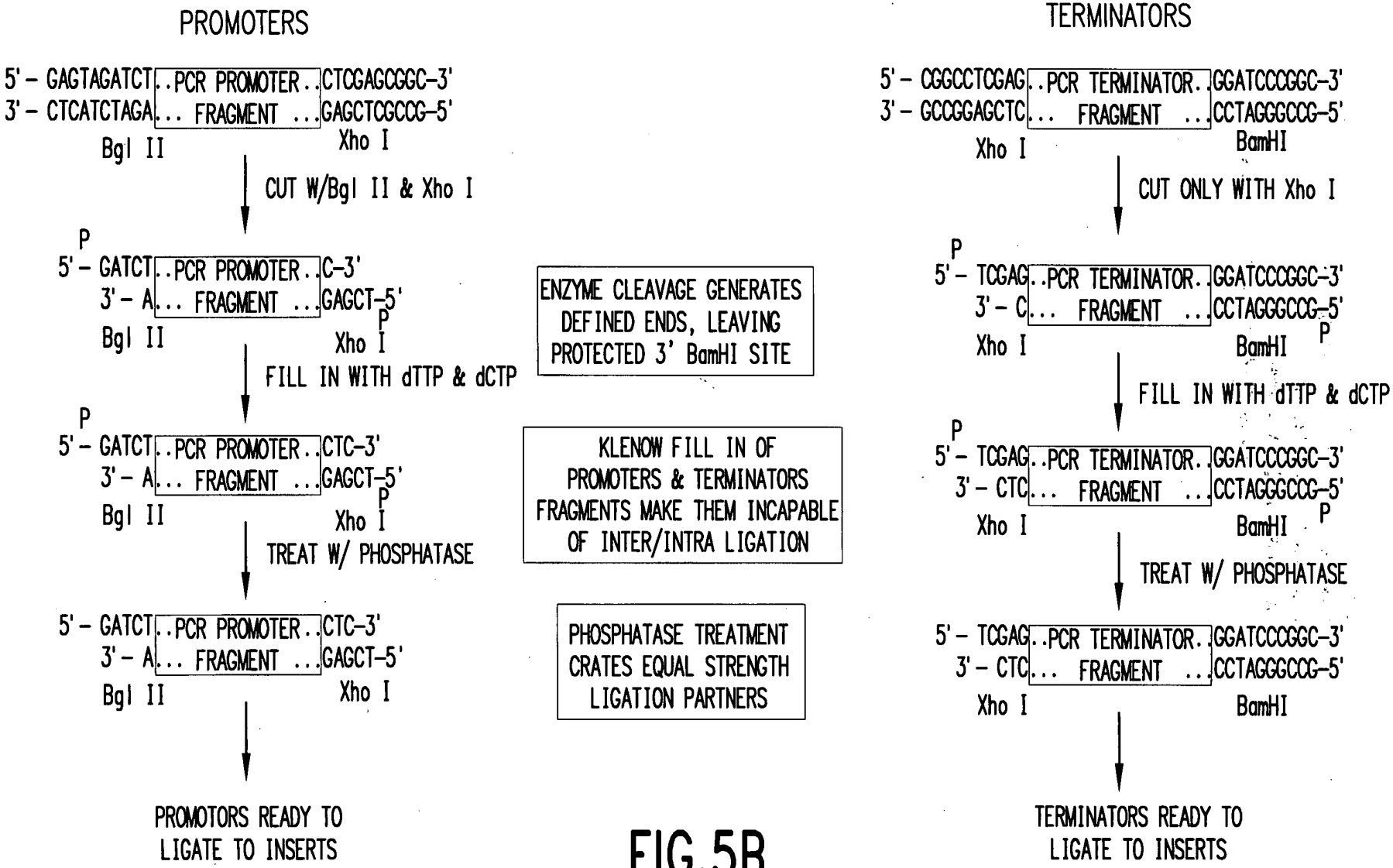


FIG.5B

APPROVED BY DRAFTSMAN | O.G. FIG. | CLASS | SUBCLASS

FIRST STRAND cDNA SYNTHESIS PREPARED WITH Not I CONTAINING poly-dT PRIMER AND 5-^{M}dCTP, AFTER 2nd STRAND SYNTHESIS, MODIFIED BamHI ADAPTERS ARE ADDED & cDNA IS DIGESTED WITH Not I, GIVING DIRECTIONAL cDNA GENE INSERTS

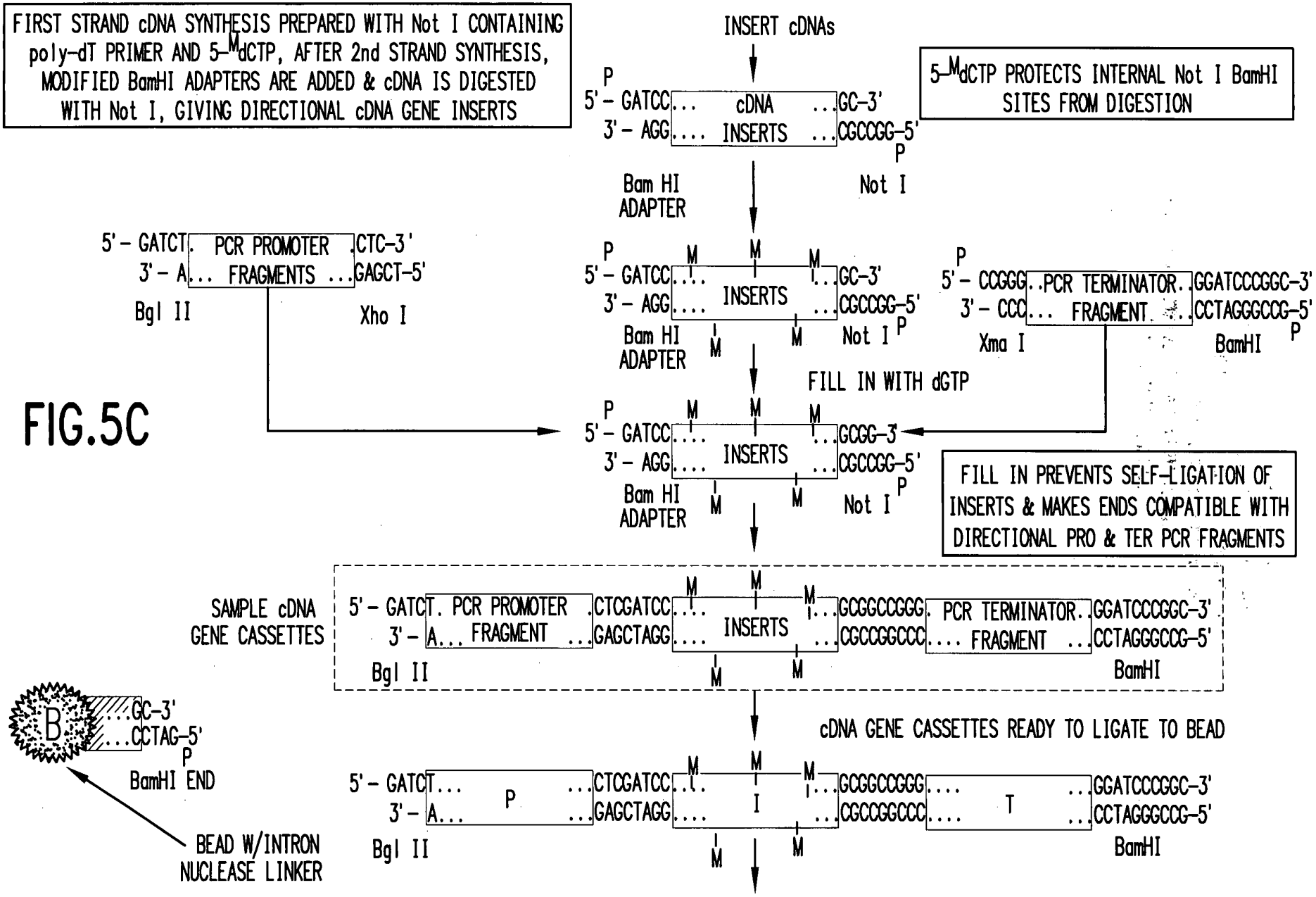
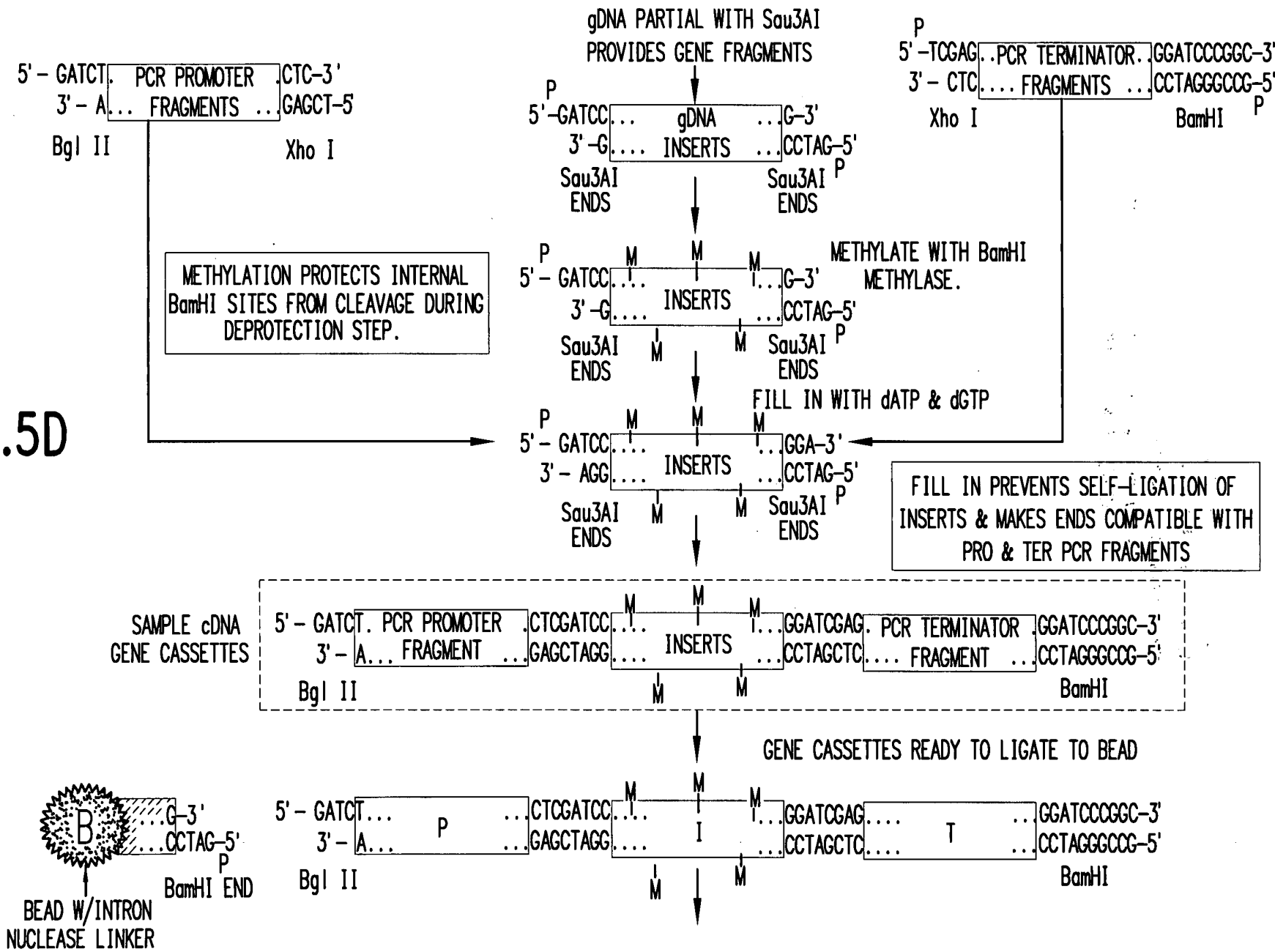


FIG.5C

FIG.5D



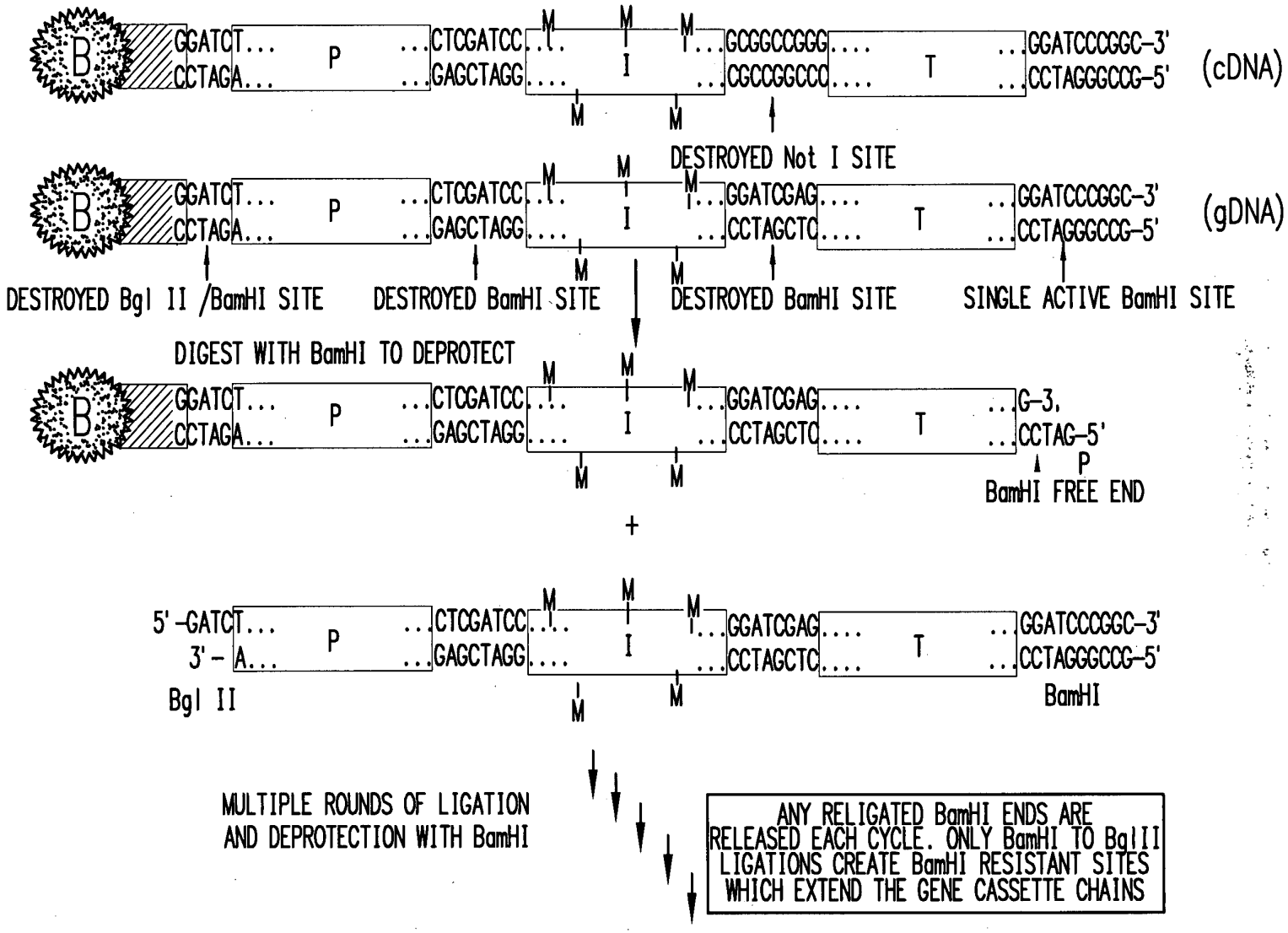


FIG.5E

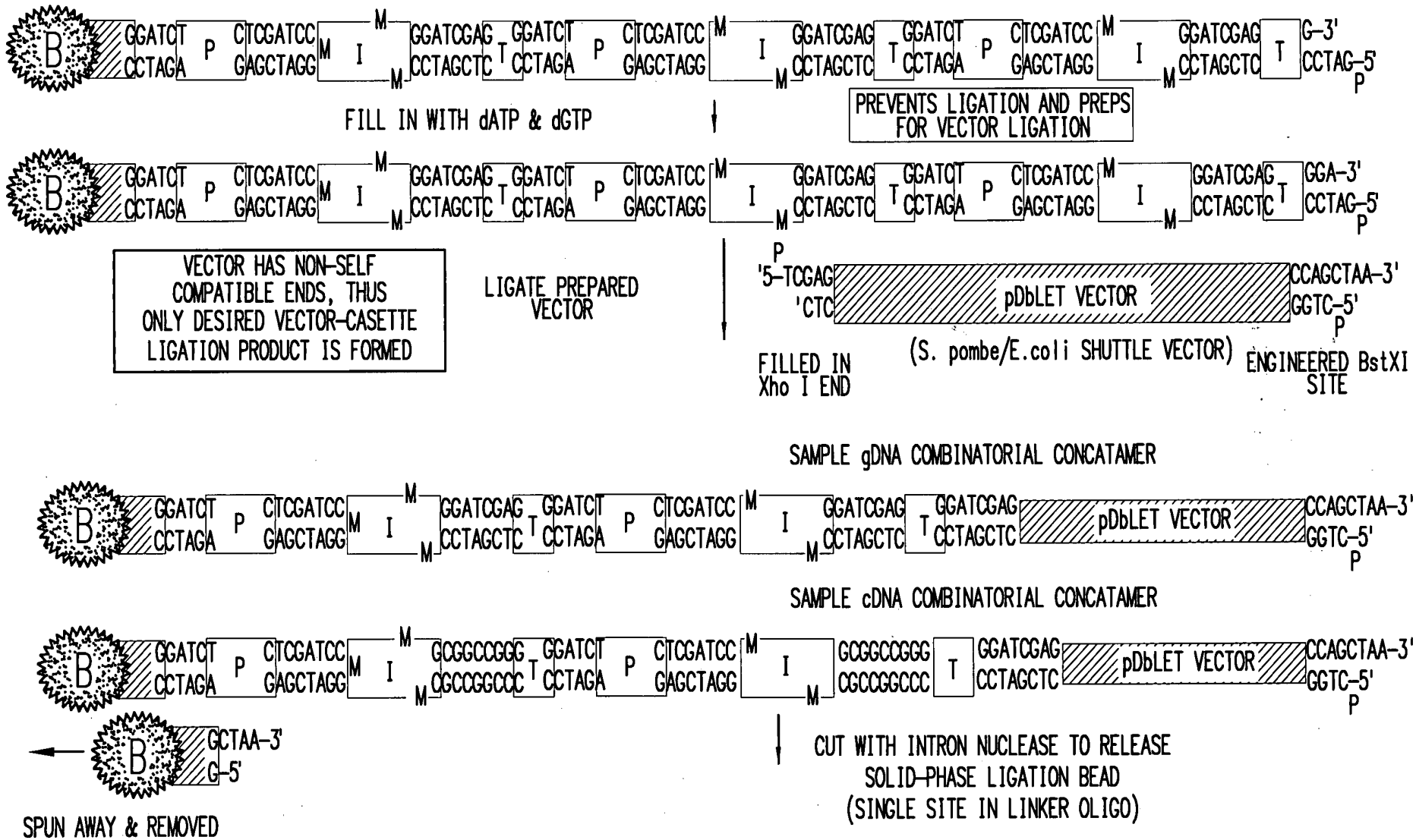
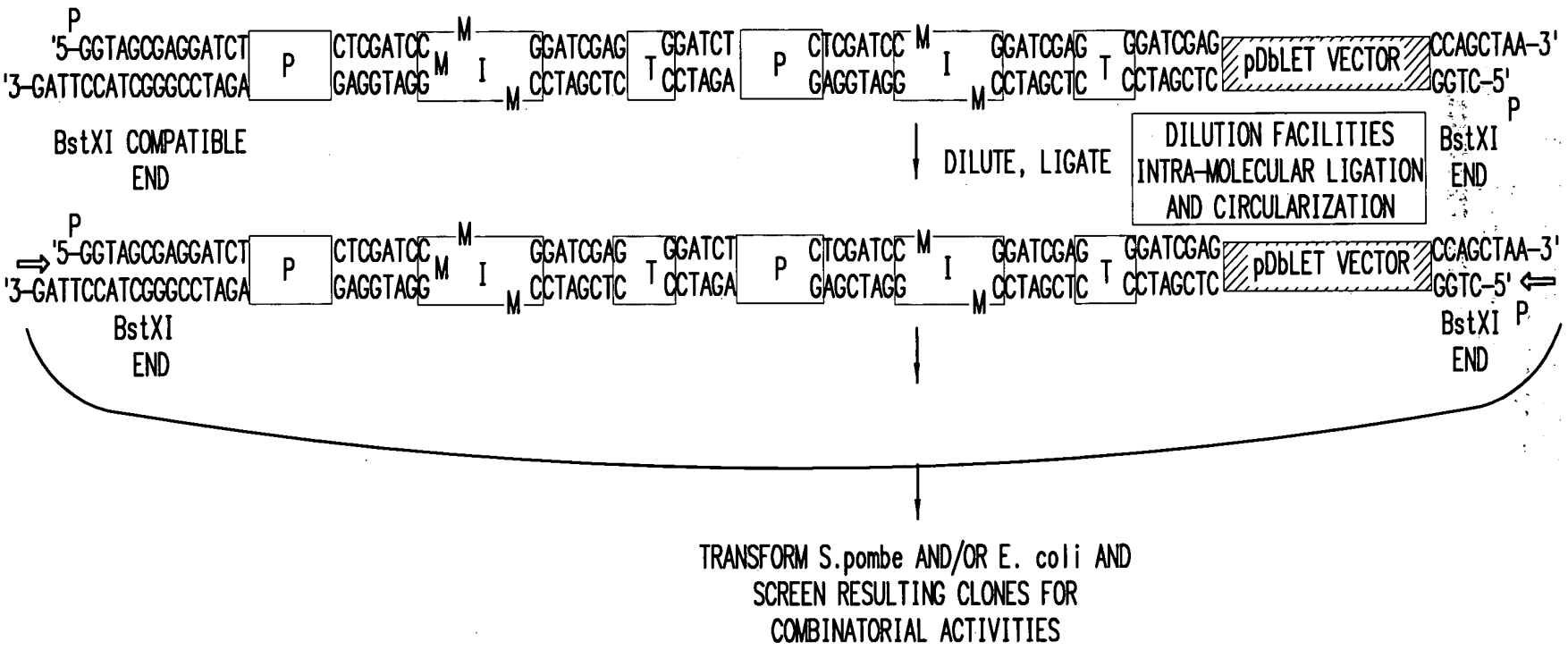

B
GGATCT
CCTAGA
P
CTCGATCC
GAGCTAGG
M
I
M
GGATCGAG
CCTAGCTC
T
FILL IN WITH dATP & dGTP
PREVENTS LIGATION AND PREPS FOR VECTOR LIGATION
G-3'
CCTAG-5'
P
GGA-3'
VECTOR HAS NON-SELF COMPATIBLE ENDS, THUS ONLY DESIRED VECTOR-CASETTE LIGATION PRODUCT IS FORMED
LIGATE PREPARED VECTOR
'5-TCGAG
'CTC
pDbLET VECTOR
CCAGCTAA-3'
GGTC-5'
FILLED IN Xho I END
(S. pombe/E.coli SHUTTLE VECTOR)
ENGINEERED BstXI SITE
SAMPLE gDNA COMBINATORIAL CONCATAMER
SAMPLE cDNA COMBINATORIAL CONCATAMER
GCGGCCGGG
CGCCGGCCC
GCTAA-3'
G-5'
SPUN AWAY & REMOVED
CUT WITH INTRON NUCLEASE TO RELEASE SOLID-PHASE LIGATION BEAD (SINGLE SITE IN LINKER OLIGO)

FIG.5F

10



TRANSFORM S.pombe AND/OR E. coli AND
SCREEN RESULTING CLONES FOR
COMBINATORIAL ACTIVITIES

FIG.5G

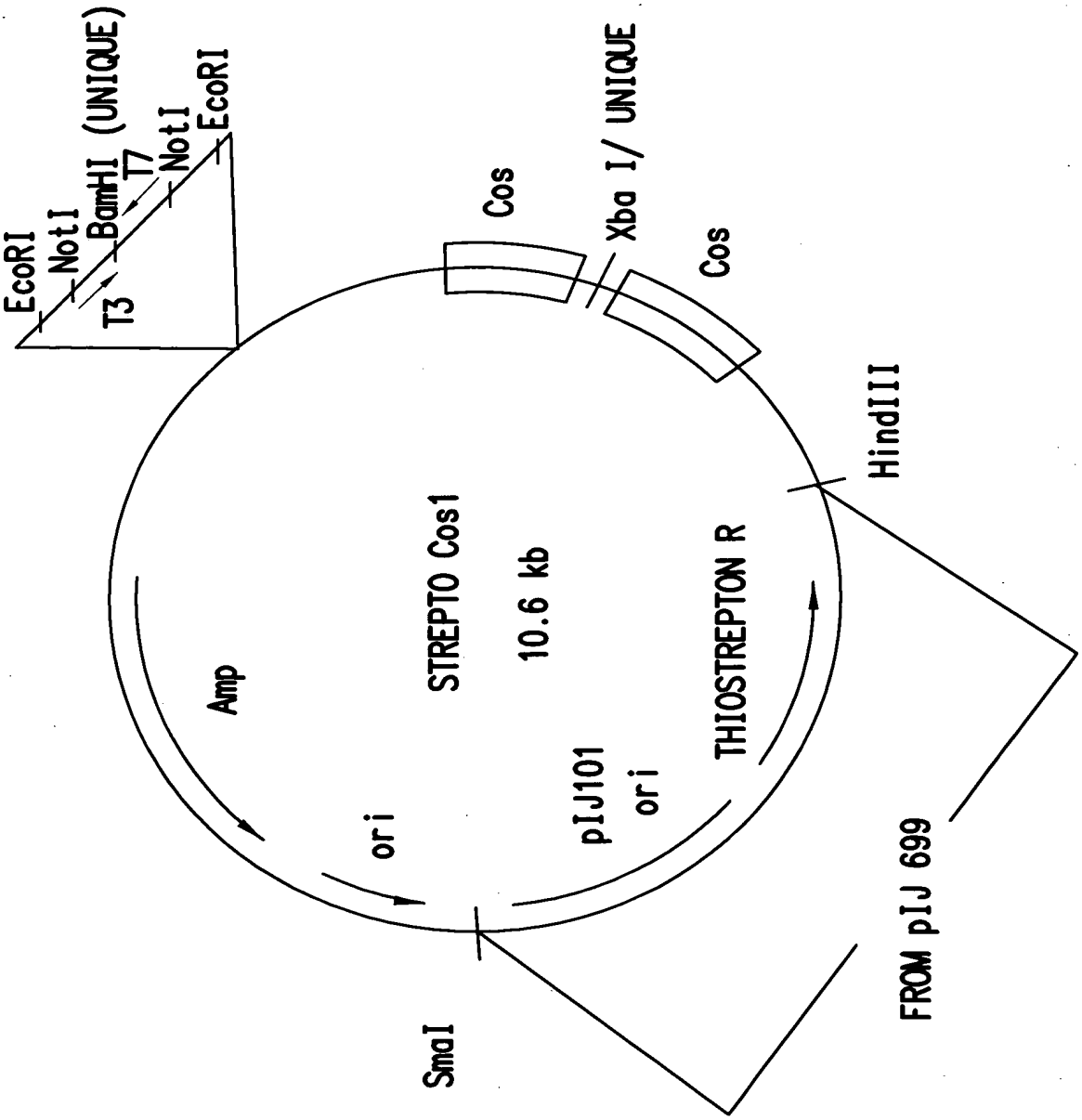
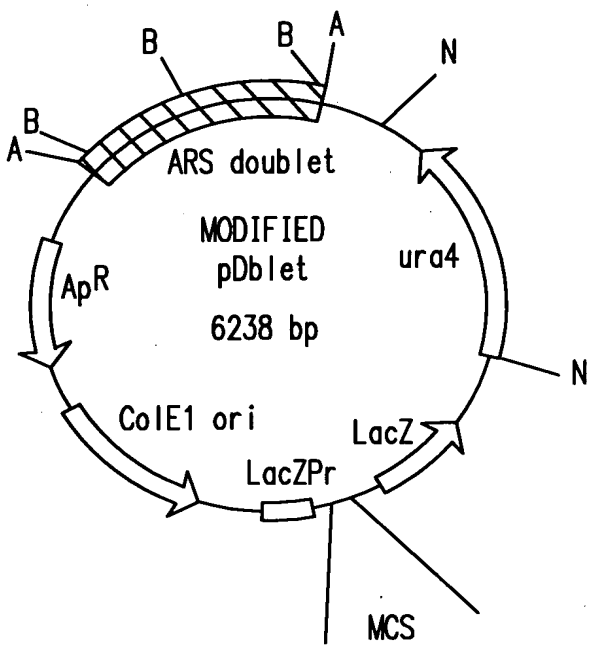


FIG.6A



MCS = SacI–NcoI–BstXI–NotI–Xba...

FIG.6B

5' CCTAGCCATGGCCACCTAACTGGGATCGC 3'

3' TCGAGGATCGGTACCGGTGGATTGACCCTAGCGCCGG 5'

SacI NcoI BstXI NotI END

FIG.6C

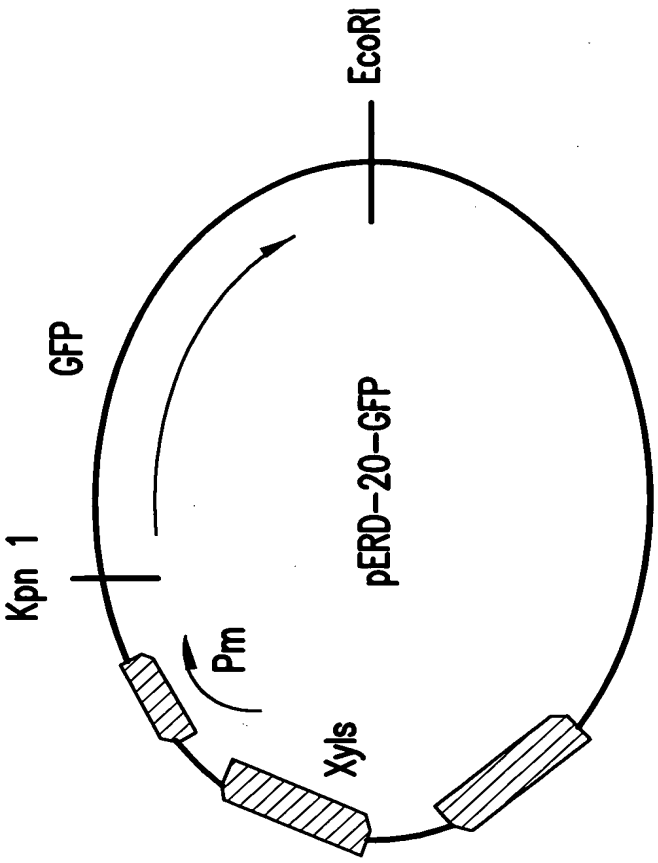


FIG.7

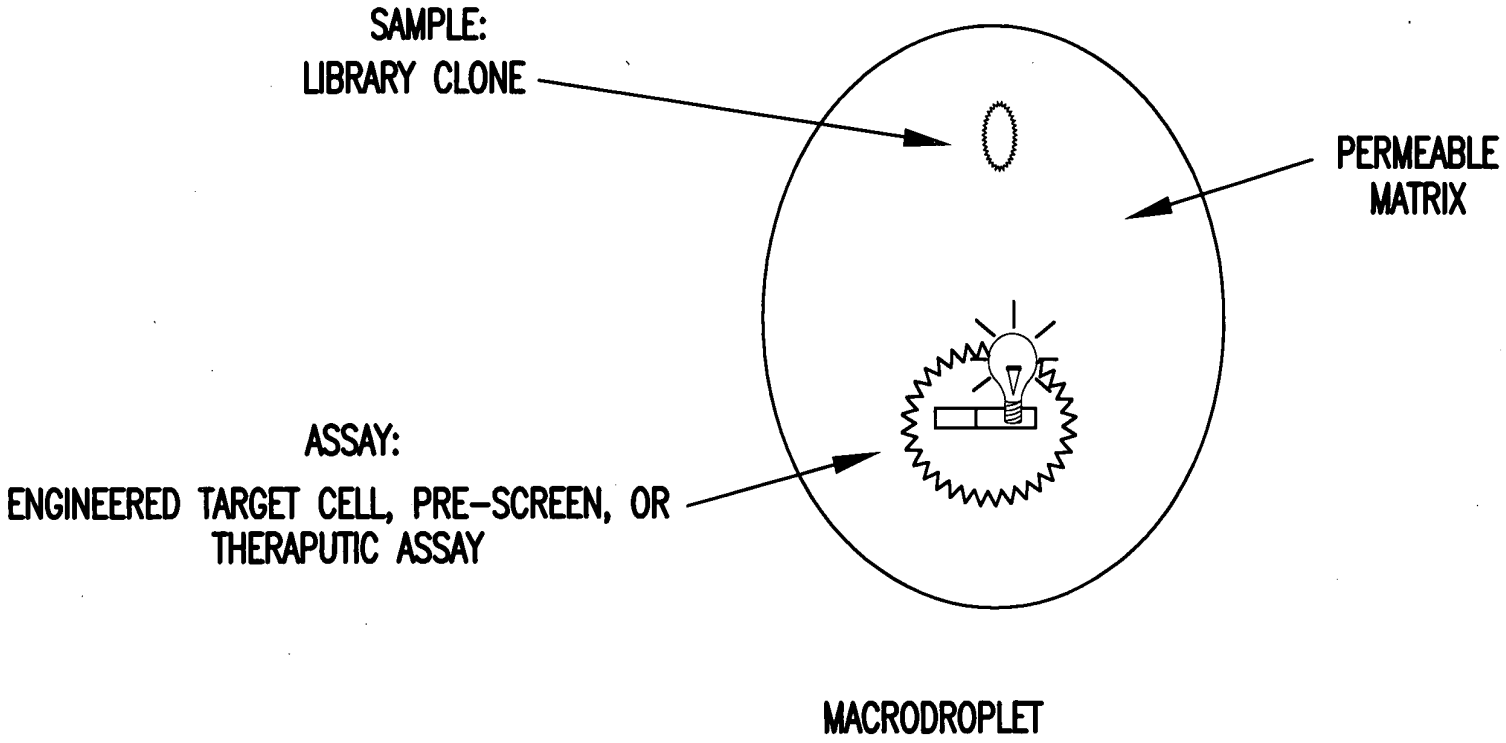

SAMPLE:
LIBRARY CLONE
PERMEABLE
MATRIX
ASSAY:
ENGINEERED TARGET CELL, PRE-SCREEN, OR
THERAPUTIC ASSAY
MACRODROPLET

FIG.8

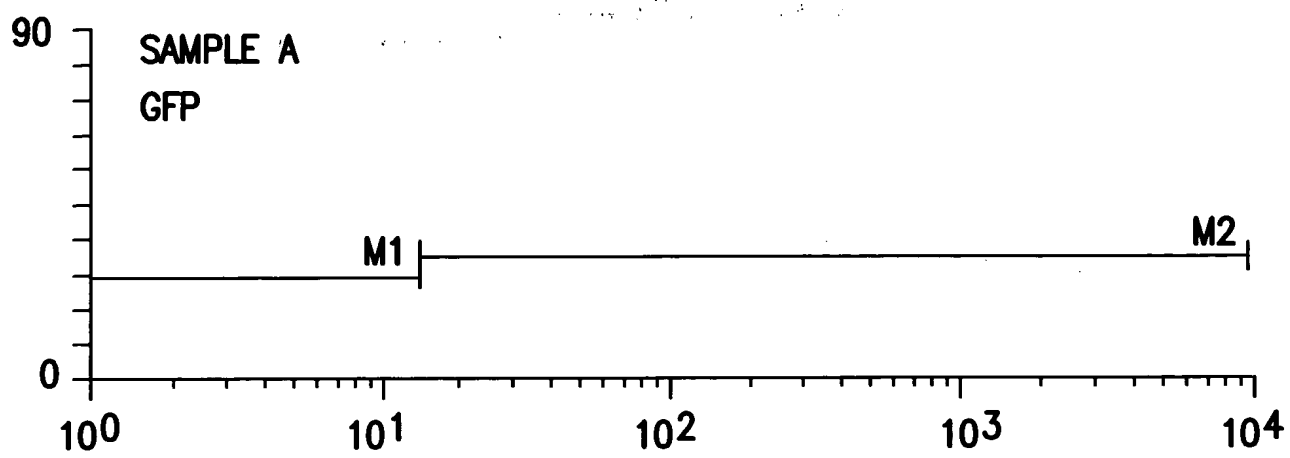


FIG.9A

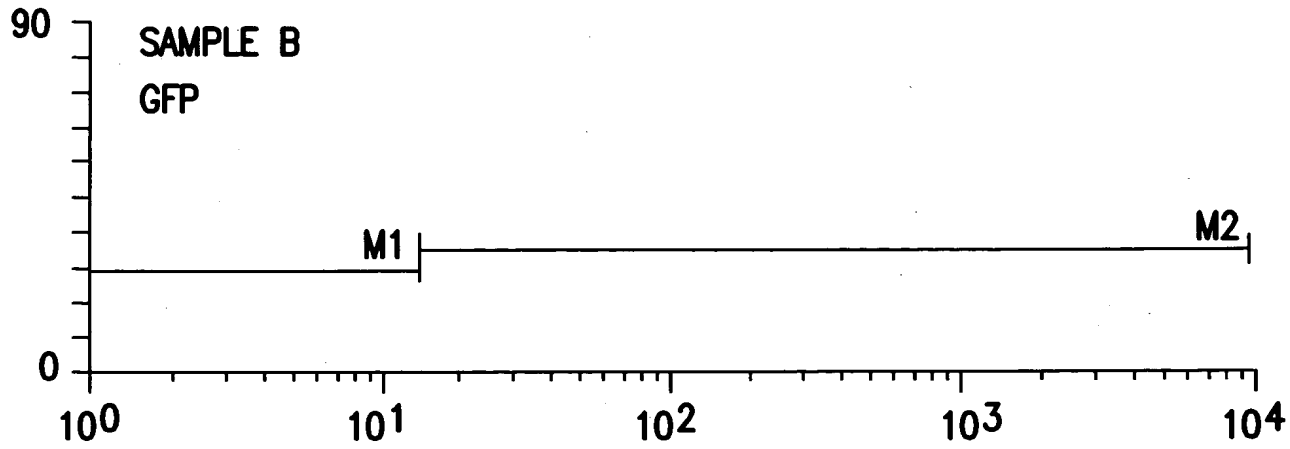


FIG.9B

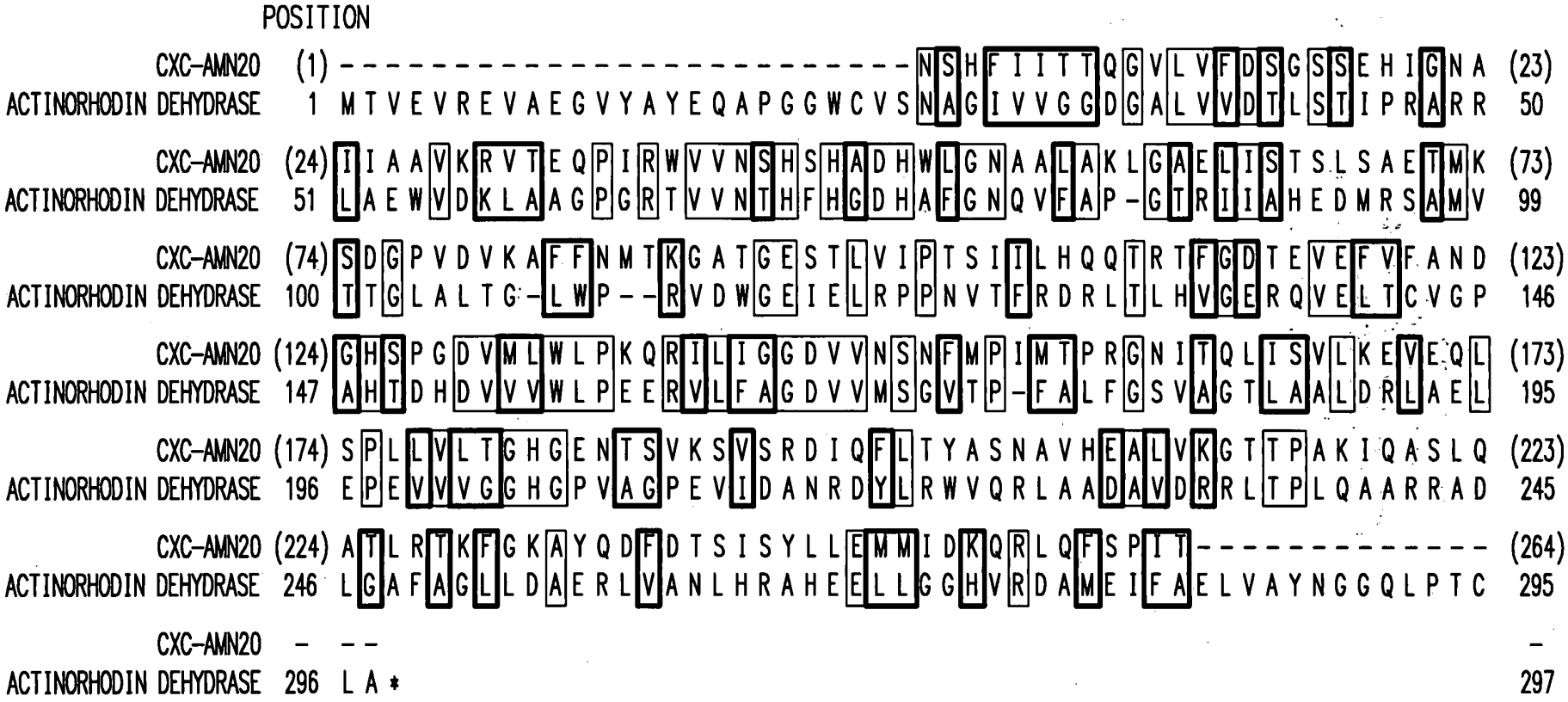


FIG.10

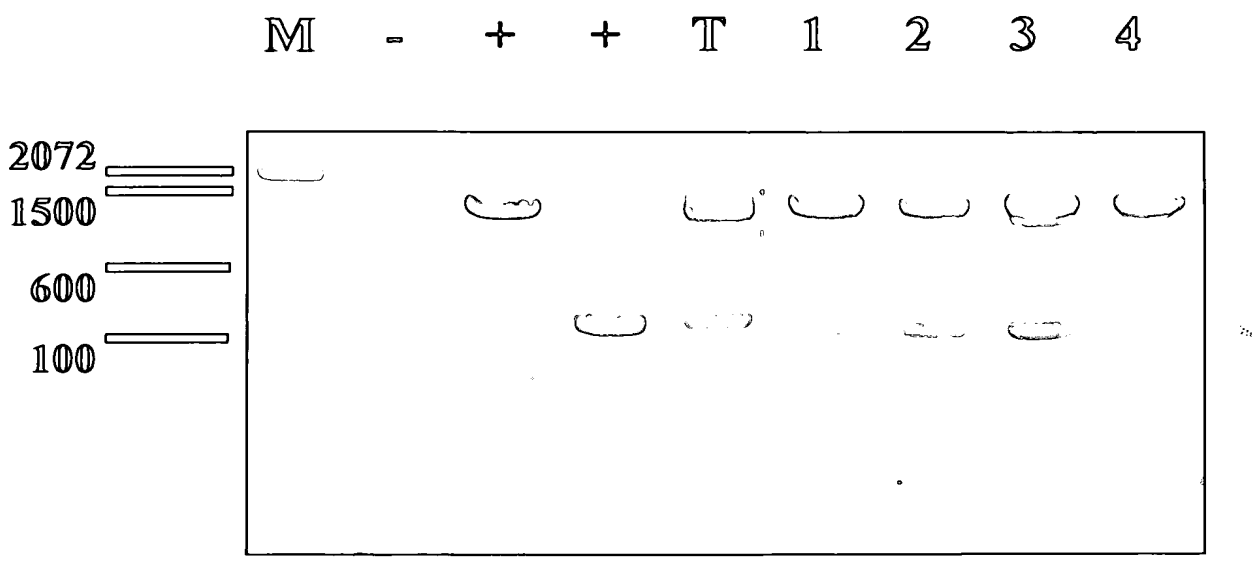


FIG. 11

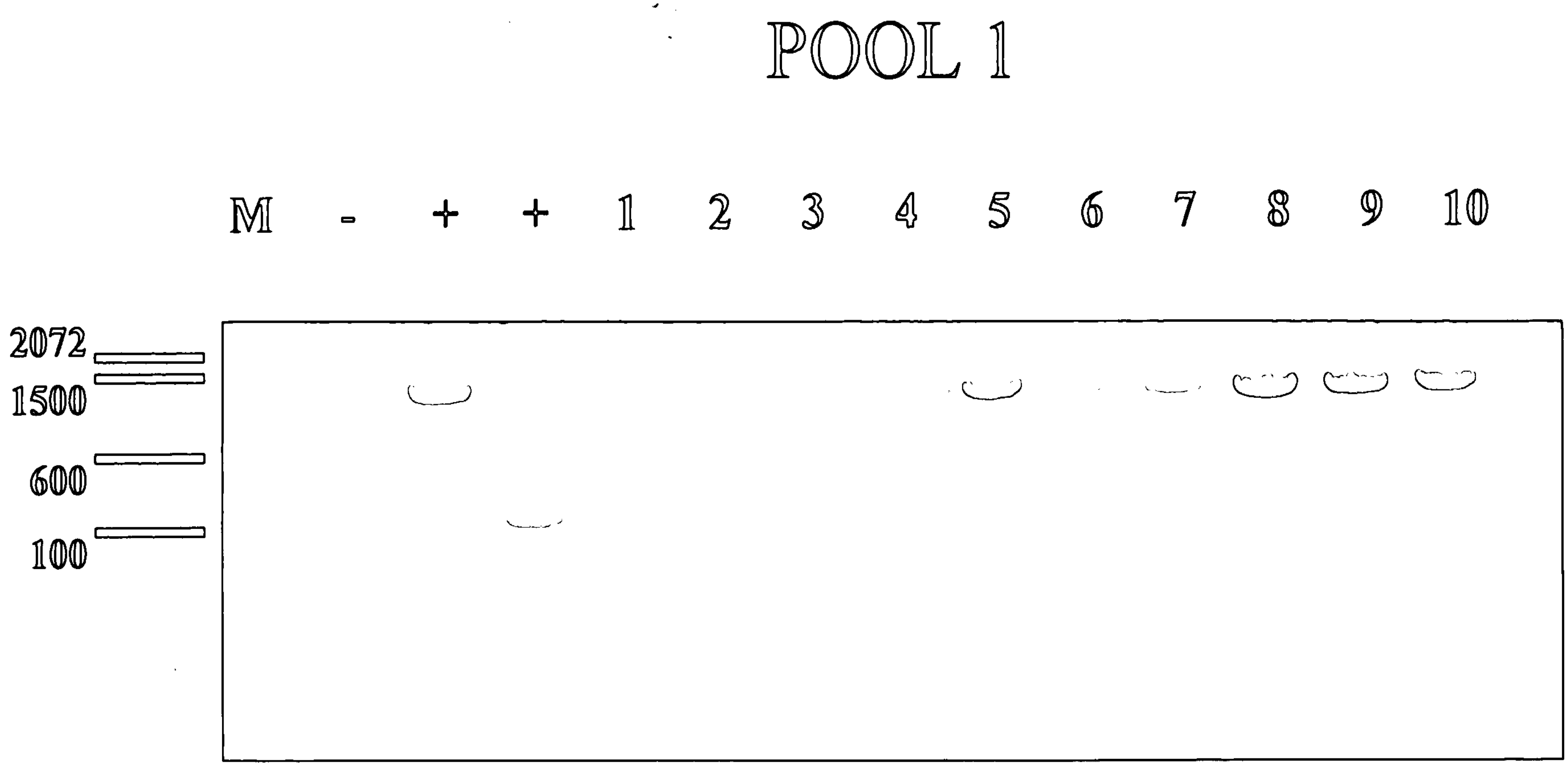


FIG.12A

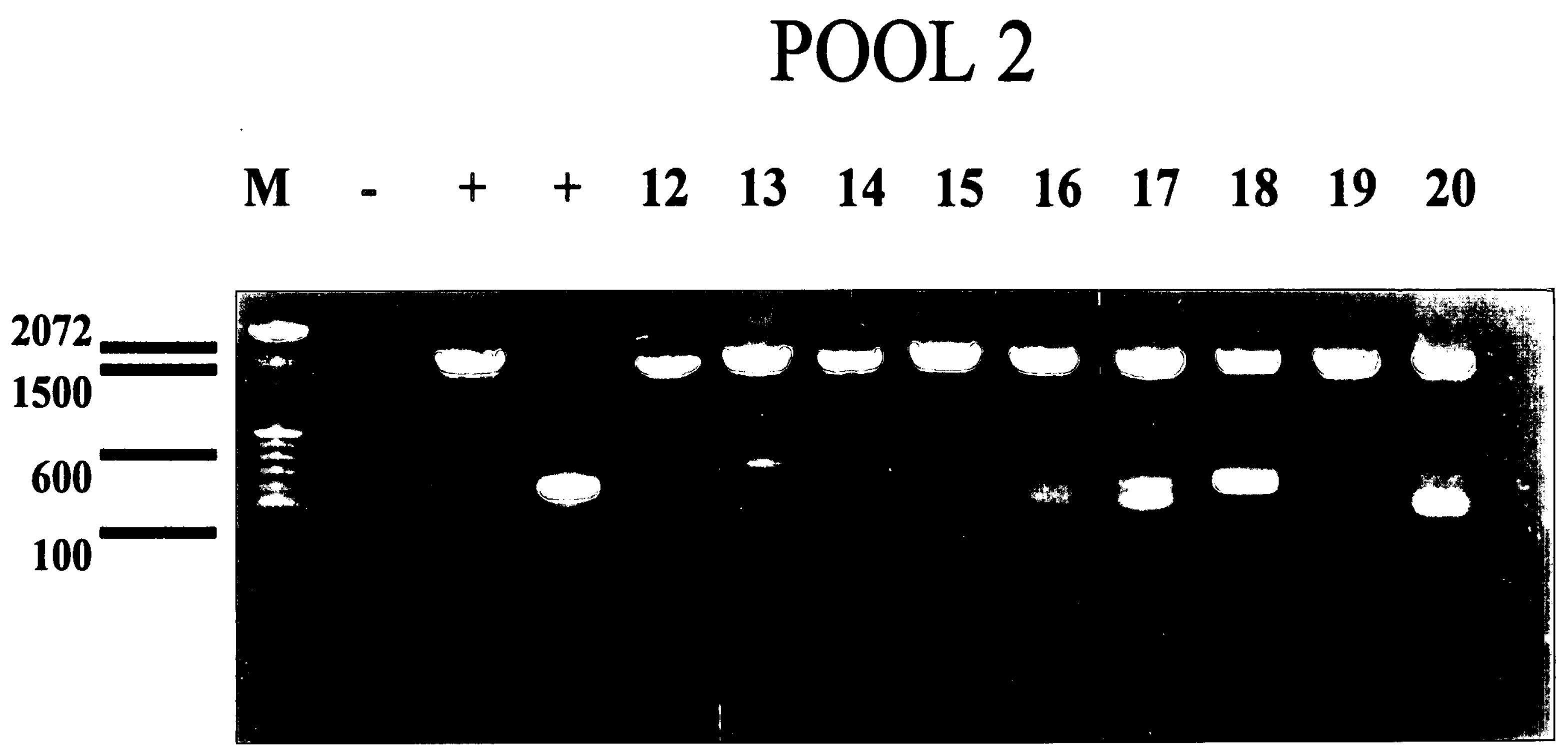


FIG.12B

POOL 3

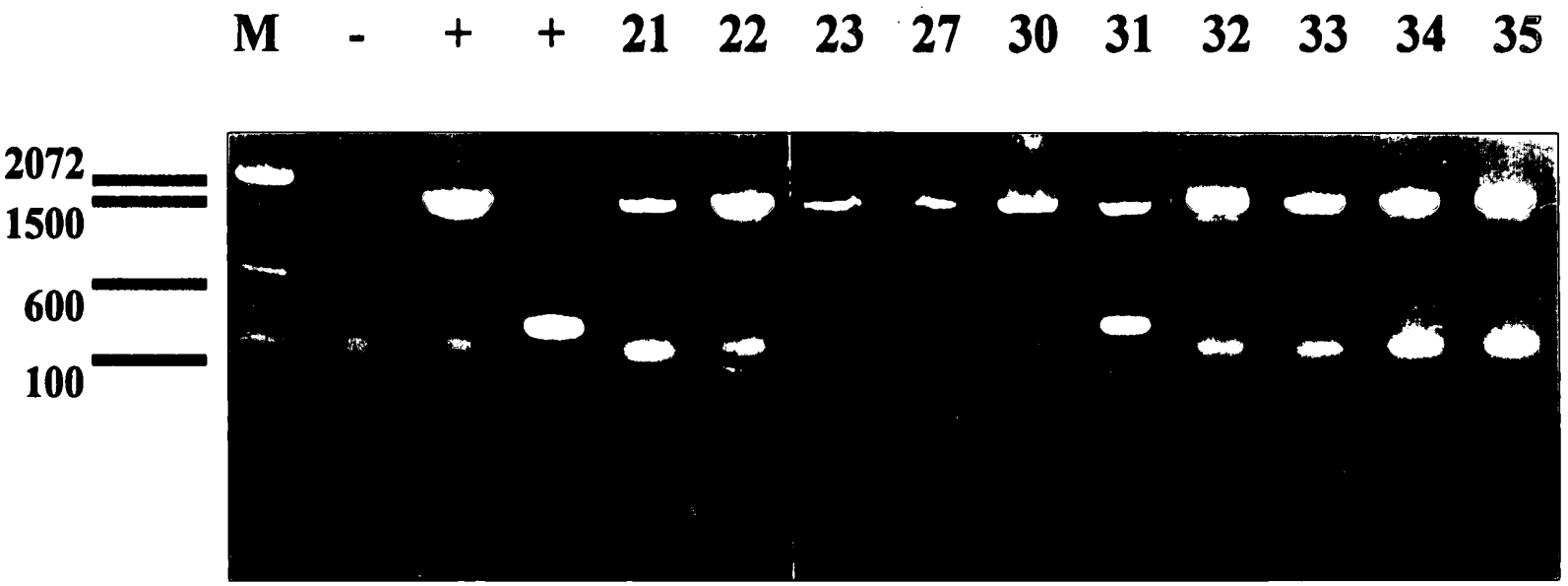


FIG.12C

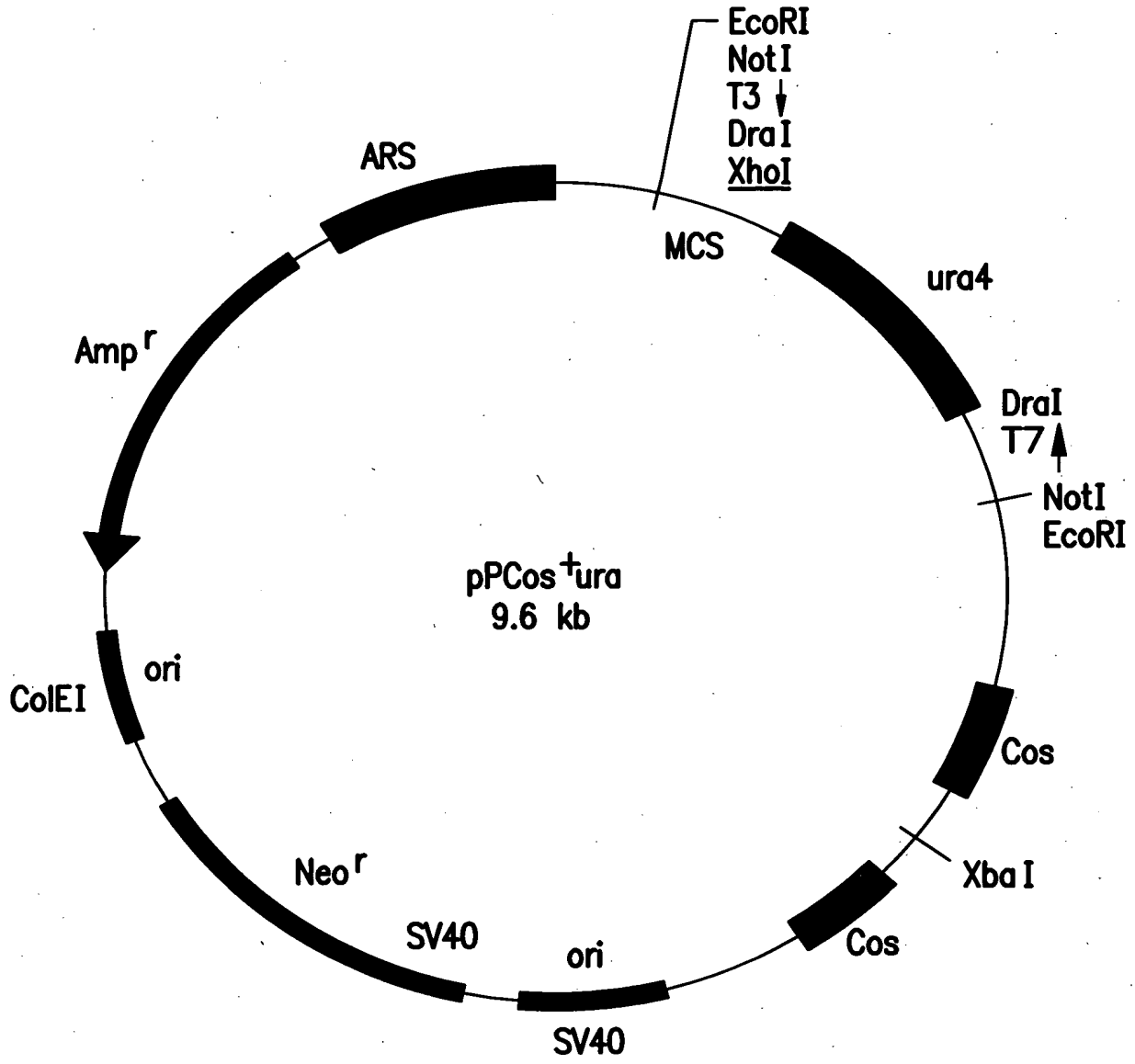


FIG.13

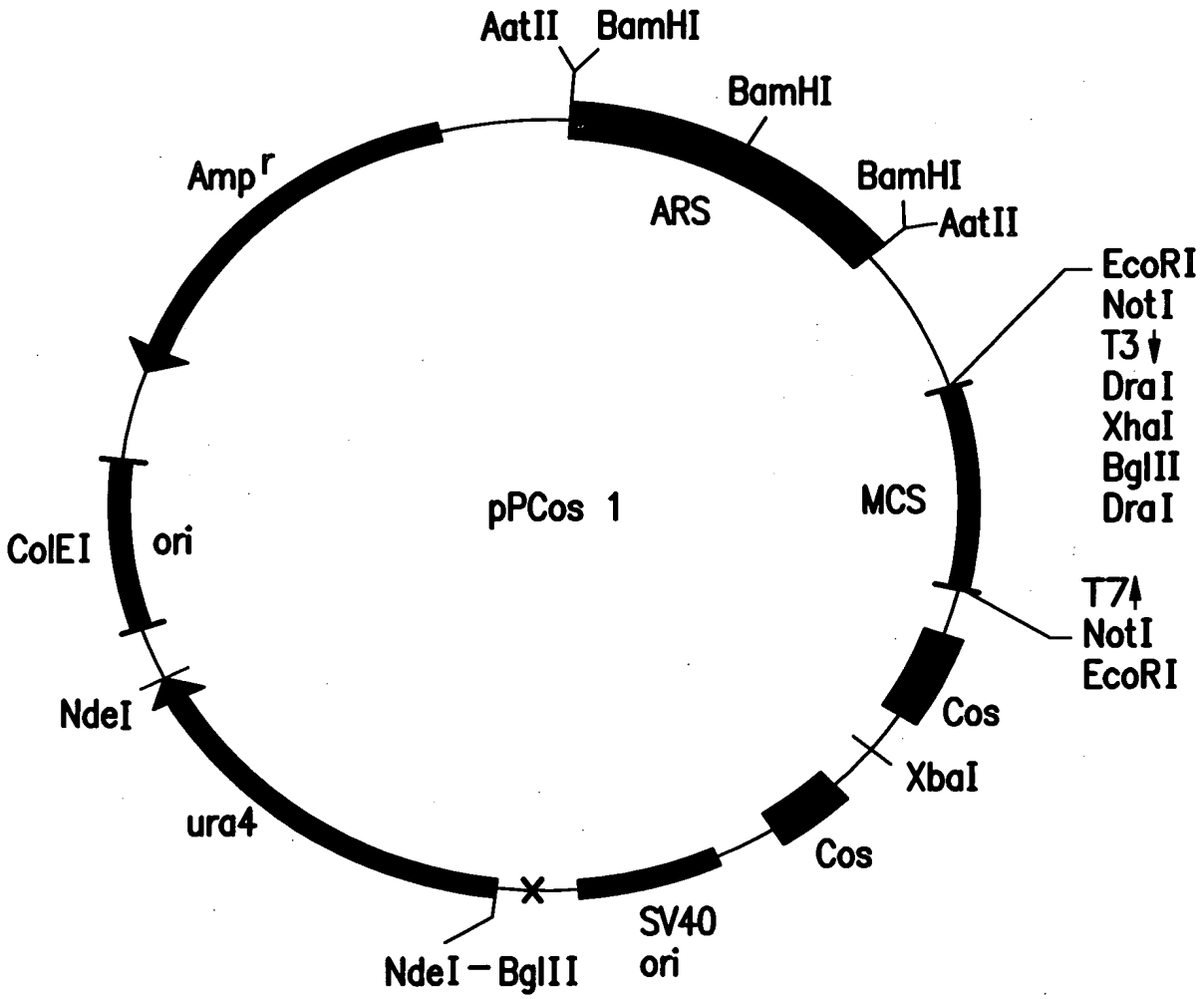


FIG.14